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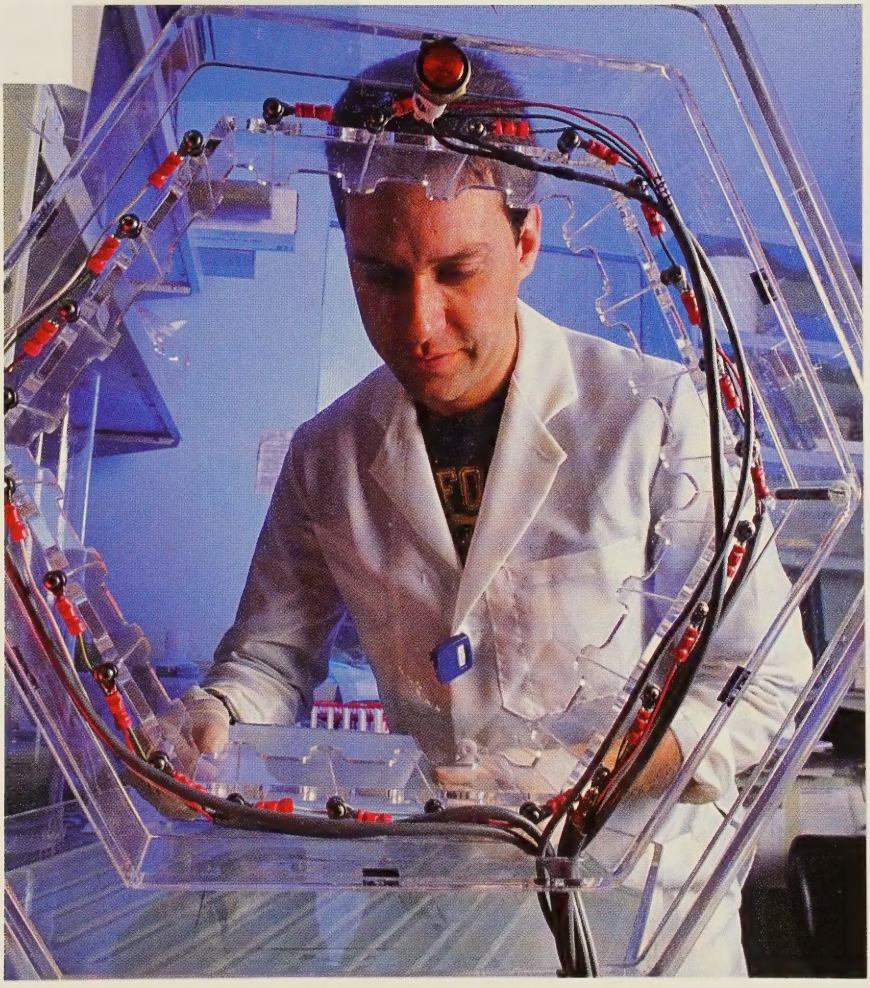
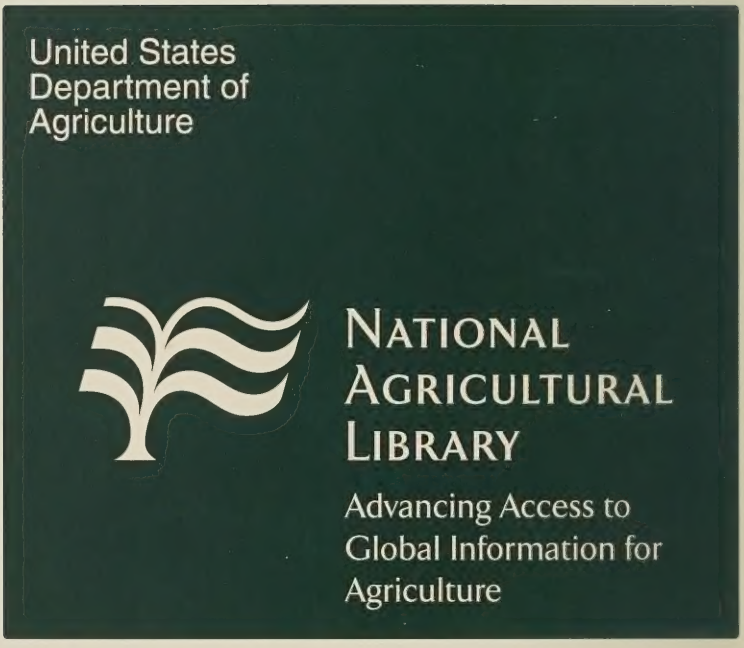
Solving Agricultural Problems With Biotechnology





Cover
At the Agricultural Research Service's Plant Gene Expression Center in Albany, California, a postdoctoral research associate separates large DNA fragments on a pulsed field electrophoresis apparatus. The goal of this work is to establish a physical method of isolating male sterility genes.

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Solving Agricultural Problems With Biotechnology

by the Biotechnology Matrix Team of the National Program Staff, Agricultural Research Service, U.S. Department of Agriculture

Modern biotechnology creates unprecedented opportunities to study and understand basic life processes and to modify and regulate them precisely with a range of techniques that were undreamed of a generation ago. The potential of biotechnology in medicine has received much attention, deservedly so. Just as important to the future prosperity of civilization is the potential for advances in agriculture.

Biotechnology promises to improve agricultural productivity; decrease our dependence on potentially harmful chemicals—pesticides, fertilizers, and antibiotics; improve safety and quality of agricultural products; decrease our dependence on petroleum as a basis for plastics and industrial chemicals; and enhance our ability to produce food on marginal lands.

The Agricultural Research Service (ARS), principal research agency of the U.S. Department of Agriculture, is a world leader in using biotechnology to help solve the increasingly complex problems of today's agriculture.

The ARS biotechnology toolbox includes every known method for investigating and manipulating organisms at the molecular level and some methods that ARS scientists are developing to meet the particular needs of their research missions. These tools encompass a broad range of new and traditional techniques.

Biotechnology Defined

ARS is a Federal research agency with various kinds of accountability and strictly adheres to established environmental regulations and biosafety guidelines in its research, especially when it comes to the production of new or changed organisms. The agency defines the term "biotechnology" as the "use of living organisms, cells, subcellular organelles, and/or parts of those structures, as well as the molecules, to effect biological, chemical, or physical changes." In ARS, this accounts for more than 54 million dollars (10 percent) of the budget for research.

ARS Research Areas Using Biotechnology

The ARS mission is to solve technical food and agricultural problems of broad scope and high national priority in conservation of natural resources, crop production and protection, animal production and protection, postharvest quality and use, human nutrition, and integration of agricultural management systems. Biotechnology has a larger role in research on field and horticultural crops, livestock, and postharvest technology than in the other areas, but it is also used in research on human nutrition and resource conservation. In these five areas, ARS conducts biotechnology research to help in—

Conservation of natural resources

- Reducing and eliminating effects of natural and artificial pollutants in soil and water.
- Adapting plants to marginal growing conditions.

Crop production and protection

- Developing cereals, forages, fiber crops, and fruits and vegetables that are—
  - Highly adapted to environmental stresses such as drought, cold, heat, and toxic soil minerals.
  - Tailored to have the best possible quality in essential nutrients and other desirable attributes.
  - Able to fix (convert to usable form) atmospheric nitrogen, minimizing the need for adding nitrogen fertilizer.
- Increasing plant resistance to diseases, insects and other arthropods, nematodes, weeds, and other pests.
- Using biochemical and genetic engineering to enhance the effectiveness of beneficial insects, parasites, viruses, bacteria, and other biological and biologically based methods of controlling plant pests.

Animal production and protection

- Developing more effective diagnostic tests for costly diseases of beef and dairy cattle, pigs, sheep, chickens, and turkeys.
- Producing new and more reliable preventive measures against livestock diseases.
- Improving the effectiveness of biocontrol organisms, or their products, used against livestock insects, ticks, and other pests.

Postharvest quality and use

- Improving the safety and quality of processed and unprocessed foods.
- Enhancing nonfood uses of farm products to increase their market value.
- Developing new uses for farm products, especially those often producing surpluses.

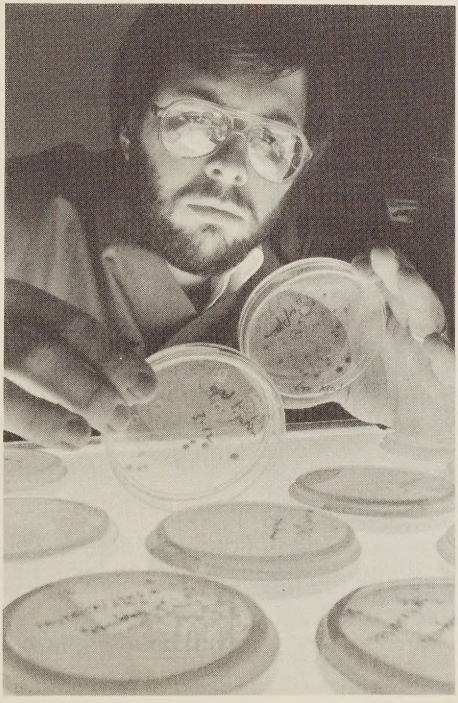
Human nutrition

- Determining just what nutrients the human body needs in what amounts and combinations.

Management systems

Though not, of course, actually using biotechnology tools, ARS systems researchers are involved in biotech research. They're developing management systems to handle the enormous quantities of data being generated in biotechnology work. These include integrated data systems—such as information on gene banks—and mathematical modeling of such factors as weather, genetic makeup, and physiology to enhance plant and animal growth.

Cleaning Up Soil With Tailored Bacteria



At the ARS Pesticide Degradation Laboratory in Beltsville, Maryland, a microbiologist checks petri dishes in which he's growing bacteria that have been genetically engineered to enhance their natural ability for breaking down toxic wastes. Genes that encode enzymes that degrade certain pesticides were cloned from the DNA of two soil bacteria—*Achromobacter,* which degrades carbofuran, and *Flavobacterium,* which speeds up degradation of coumaphos. Carbofuran and coumaphos are potent insecticides; the more rapidly they can be broken down, the less likely they are to seep into soil and groundwater. (0386X501-35)

Biotechnology Research

Scientists use biotech tools to study, manipulate, and modify specific organisms. Most of the techniques described in this book can be used for both diagnosis and manipulation. And biochemical engineering and bioregulation research are biotechnologies that use many of the individual tools.

What follows is an overview of ARS biotechnology research with examples of ongoing projects and recent accomplishments. Detailing all the techniques and all the projects isn't possible here. For further general program information, call or write:

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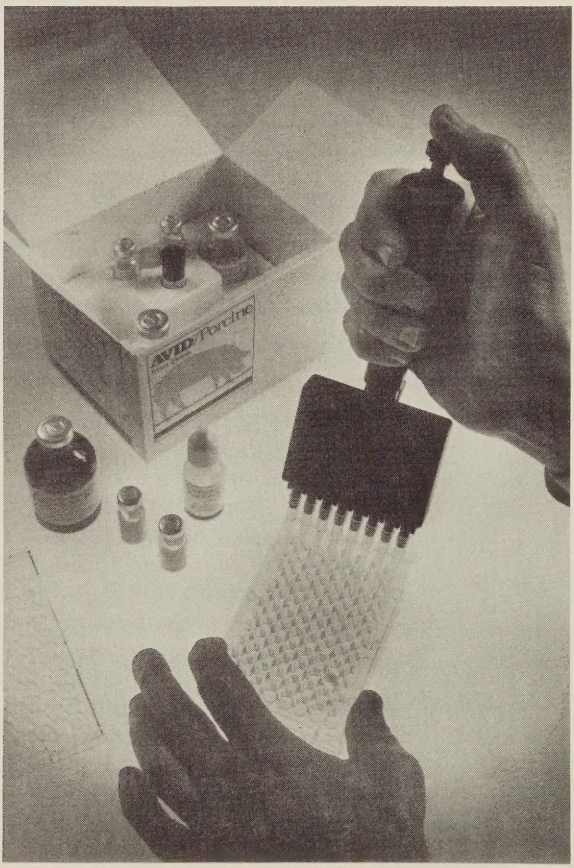


A commercially produced trichinosis test kit based on ARS research using monoclonal antibodies. (88BW0063-6)

Technical information on current and recently completed research projects is available through CRIS (Current Research Information System) Online, a database accessible through Dialog Information Services, a commercial retrieval service. Many libraries, including the National Agricultural Library in Beltsville, Maryland, will do CRIS searches, often for a fee. The CRIS database includes abstracts, scientist names and locations, and lists of recent publications on nearly all current agricultural and forestry research funded in whole or part by the Federal Government. It is also published on commercial CD-ROM disks that may be available for use at some institutional libraries.

ARS has its own online database, called TEKTRAN. Subscriptions are offered on a first-come-first-served basis to organizations (such as commercial firms, universities, and government agencies). The research results described in the TEKTRAN database have been peer-reviewed but not yet published. To ask about access to TEKTRAN, call the ARS National Technology Transfer Coordinator at (301) 344-4045.

Monoclonal Antibodies

Monoclonal antibody technology is used to produce large amounts of nearly identical antibodies, which can single out specific antigens (chemicals or microorganisms that stimulate antibody production).

ARS scientists are developing and using monoclonal antibodies as basic research tools in several kinds of projects, such as the discovery, study, and enhancement of biocontrol agents; study and manipulation of plant bioregulation mechanisms; and especially the detection of organisms that cause plant and animal diseases, location of specific proteins, and development of vaccines that capitalize on the animal's immune-system response and are more specific than traditional (trial-and-error) vaccines.

Diagnostic tests for plant and animal diseases

One of the more useful features of monoclonal antibodies is that they "recognize" specific immunogens, substances that trigger immune responses. If an immunogen is present, the monoclonal antibody should find it. So developing monoclonal antibodies to immunogens associated with a particular disease is a means to a highly specific method of disease diagnosis.

In 1984, an ARS parasitologist in Beltsville, Maryland, used monoclonal antibodies to develop a blood test that is 90 percent accurate in detecting trichinosis in pigs. (Trichinosis is caused by the

parasitic trichina worm; in the United States, only about 1 in 1,000 pigs is believed to be infected, and humans can avoid contracting the disease by adequately cooking or freezing their pork.)

The test was originally developed as a research tool to be used in projects aimed at eliminating trichina infections in domesticated swine in the United States. But the test had obvious commercial applications as well; the technology was patented and then transferred to several firms through licenses and cooperative research agreements. Commercial diagnostic kits, based on refinements in the original test, are now available for testing pigs on the farm or at the slaughterhouse.

Other diagnostic tests resulting from ARS research with monoclonal antibodies include:

- A test for active neutrophils (a kind of white blood cell) in cattle. Cattle with active neutrophils have greater disease resistance and could be used to produce more disease-resistant breeds.
- A broad spectrum diagnostic kit that will quickly detect some of the world's most damaging viruses to plants in one test. Being marketed by a commercial firm, the kit is based on an ARS-developed monoclonal antibody (patent applied for) that reacts to a site on a protein molecule common to most, if not all, potyviruses. Named after potato virus Y, potyviruses affect many important crops, including corn, soybeans, wheat, lettuce, and other vegetables and ornamentals such as tulips and Easter lilies. Seed-testing firms, nurseries, farmers, and government agencies that quarantine plants are among the kit's potential customers.

Like the trichinosis test kit, many of these diagnostic tests are offshoots of research aimed at learning more about immune response and the processes of disease development so that scientists can enhance disease resistance through genetic engineering, breeding, and other means.

DNA Hybridization and DNA Probes

DNA—or deoxyribonucleic acid, and RNA—ribonucleic acid—are molecules made up of chains of nucleotide bases. The sequence of these bases on the chain is the information governing an organism's form and biology.

DNA is usually double stranded; the nucleotide bases in one strand are bonded with complementary nucleotide bases in the other, forming base pairs. RNA is usually found as a single chain. A DNA molecule's two strands can be separated, and an RNA strand can bond with one of these separated DNA strands.

DNA can be cloned, separated into single strands, or labeled with radioactive isotopes or other markers. Researchers can also synthesize DNA complementary to the DNA they're interested in and use it as a detection probe. Complementary DNA, or cDNA, is produced from an RNA sequence, usually messenger RNA, using an enzyme called reverse transcriptase. RNA fragments can also be used as probes in work with nucleic acid hybridization.

The hybrid of the DNA/DNA or DNA/RNA strands may be joined along the entire length of the strands, or only partly, depending on the match of the individual nucleotide bases. How much the cDNA strand and its complement match can tell scientists a great deal about, for example, how closely related two species happen to be or the locations of particular genes.

Like monoclonal antibodies, DNA probes are used as diagnostic tools in many research projects directed at understanding and eventually controlling basic biological processes in crop plants and farm animals and in the pests and diseases that diminish their economic value.

And as with monoclonal antibodies, ARS research with DNA probes has led to more accurate diagnostic tests for certain animal diseases—leptospirosis, which causes abortions in cows, and anaplasmosis, which infects red blood cells in cattle, causing anemia. Similarly, ARS plant pathologists and geneticists have developed several diagnostic tests using DNA probes to detect viruses and viroids that cause plant diseases such as potato spindle tuber disease.

Recombinant DNA

Molecular biologists construct recombinant DNA molecules by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell. The result is a new genetic combination. Recombination occurs naturally; it is the key to genetic diversity. But of the billions of recombinations that occur in nature each day, few are successful and even fewer have a practical value. Laboratory recombination controls and channels this natural process.

Recombinant DNA technology can be used as a diagnostic tool and as a means for creating specific vaccines. In one project, ARS researchers are using it to develop vaccines to protect cattle against cattle grubs and other arthropod pests. Such a vaccine is being tested now; if successful, it would be the first recombinant DNA vaccine to protect an animal against an insect pest.

Gene Mapping and Sequence Analysis

Gene mapping determines how genes are arranged on a chromosome. ARS scientists are mapping genes for several organisms as part of genetic studies essential to sophisticated cross-breeding of plants and animals.

Especially useful tools for mapping genes are restriction enzymes. Because they cut DNA strands at predictable places, restriction enzymes are commonly used to create DNA restriction fragments. A restriction fragment is a piece of DNA cut by a restriction enzyme. A given type of fragment that often varies in length in the population (a "length polymorphism") defines a genetic variable. This information is particularly helpful if the restriction fragment length polymorphism (RFLP) is associated with observable differences in the organisms—such as susceptibility to disease. The techniques can be used for specific identification or differentiation of closely related microbes such as pathogenic variants.

Just one ARS-developed example of the potential of this biotechnology: Using restriction enzymes, researchers can now distinguish clearly and reliably between two look-alike species of sugarcane—*Saccharum sinense* has an additional fragment of DNA that's not found in *S. barberi*. Accurate identification of the two species is critical because they're among the six used today in producing commercial sugarcane varieties.

Knowledge gained from gene mapping can be combined with the knowledge gained from sequence analysis to give scientists much of the information they need to understand and improve a given organism. Analysis of the sequence of nucleotide bases in a particular segment of DNA gives genetic engineers a vocabulary to work with in manipulating the genetic "sentence." For example, ARS researchers at Albany, California, and colleagues in England have sequenced the DNA of two genes thought to be responsible for high-quality flour. These genes direct production of special glutenin proteins usually found in superior flour. This knowledge will be used to help improve the genetic makeup and therefore the breadmaking quality of wheat varieties.

ARS research projects using gene-mapping tools and sequence analysis range from identification and enhancement of biocontrol agents to improving diagnosis and treatment of plant and animal diseases.

One project is using gene mapping to define more precisely the genetic differences among certain insect pests of field crops (especially corn earworm and tobacco budworm and related species). Better knowledge will lead to more narrowly focused control measures, which will be more effective than current methods and less likely to damage the environment.

Gene mapping is also an important component of modern plant breeding research. ARS scientists are using information about the locations and relationships of specific genes to improve germplasm of, for example, soybeans, corn, tall fescue, and barley.

In mapping genes, scientists are learning more about what makes living things work. Most crop plants and livestock animals are genetically complex, and it will probably be many years before their genetic mechanisms are completely understood. So the most immediate payoffs are likely to come from work with simpler organisms.

ARS researchers, for instance, are using gene mapping to gain practical information about plant pathogens—organisms, such as bacteria and viruses, that cause plant diseases. Studies are concentrating on the genetic factors that determine a pathogen's virulence, or capacity to actually cause the disease, and its ability to resist antibiotics.

Another project involves DNA sequencing of genes responsible for lysine synthesis in rice; the goal is to increase production of this nutritionally essential amino acid in various cereal crops.

Genome mapping project

Whatever the technique, whatever the immediate goal, gene mapping and sequencing contribute to a complete picture of an organism's genetic makeup, or genome.

But while many mapping projects are now being carried out by Federal, State, university, and industry researchers, there has been no single major project to develop complete gene maps of the important food, fiber, industrial, and forest crops.

Yet knowledge about the genomes of these crop species is essential. We need to know the location of genes that control or influence yield, time of maturation, nutritional content, and resistance to disease, insects, and drought so that we can continue to improve crops.

To achieve this goal, the Secretary of Agriculture, Clayton Yeutter, announced, in early 1989, plans for a plant genome mapping program—a major research effort to identify the most important genes present in the major food and forest crops and to determine what the genes do and how they function. ARS has been assigned responsibility for leadership in developing the plant genome mapping project. The agency will act as a focal point for a system of interrelated Federal, State, and university research that contributes to this project.

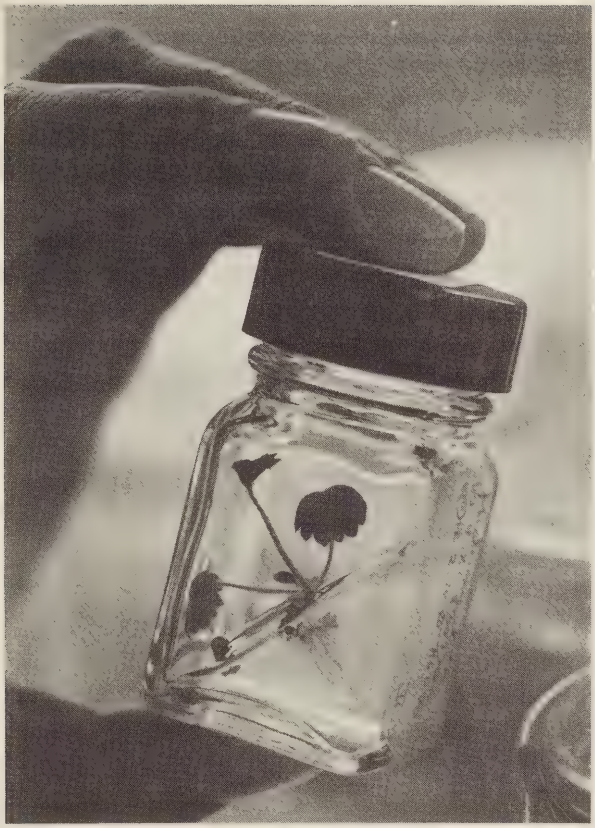
The project is likely to be even more complex than the human genome study recently begun because of the number of plant species involved. Corn alone has about the same number of nucleotide base pairs as human chromosomes—3 billion.

ARS scientists at Raleigh, North Carolina, have already made a start on mapping the corn genome. They are mapping the genes of higher yielding corn by tagging corn chromosomes with previously identified DNA sequences.

The Plant Molecular Biology Laboratory

The Beltsville (Maryland) Agricultural Research Center is the largest complex of laboratories, scientists, and support staff in ARS, making it the most prominent center of agricultural research in the world. Since its beginnings in 1910, Beltsville has contributed greatly to the continuing prosperity of American agriculture and the well-being of people everywhere, from genetic concepts that laid the foundation for modern plant and animal breeding to the creation of a standardized reference diet for use as a research tool in human metabolic studies, from developing the Beltsville Small White turkey to identifying and supplying disease-resistant wheat to plant-breeding centers around the world for the Green Revolution (a turning point in agriculture that drastically reduced world hunger).

Two of the most notable accomplishments at Beltsville came in plant biology—discovery and isolation of phytochrome, the photoreceptor that regulates many plant growth and development responses to light, and discovery and isolation of plant viroids, a new class of disease-causing particles 80 times smaller than viruses. The laboratories where these discoveries were made are the ancestors of the recently established Plant Molecular Biology Laboratory, which was installed in April 1989 in a newly remodeled and modernized building at the Beltsville center. The goals of the new lab are to identify and isolate genes that control plant metabolism, to develop reliable tools for transferring the genes, and to find ways to regrow resulting transgenic plants. Recent research accomplishments include a tissue-cultured rice with high lysine content (which leads to more and better protein) and development of a technique to transfer genes into pollen by electroporation—using split-second electric shocks to open pores in the pollen that allow entry of new genes. Other accomplishments include tissue culture of peach trees (see p. 12), in vitro root production of soybeans (see p. 16), and transfer of a corn storage protein enriched in sulfur amino acid content (see p. 13).



Tissue-cultured strawberry plantlet is one of many horticultural crops cloned to be free of disease-causing organisms and to be extra vigorous. (0386X429-25a)

Tissue Culture

Use of tissue culture enables scientists to grow the early stages of an organism in a laboratory environment. The organism is grown in vitro (literally "in glass"). "Tissue culture" is a general term covering all forms of in vitro culture: The organism can be started from tissue (an aggregate of similar cells) or from a single cell, from an embryo, and from other sources such as the anther (part of the male reproductive organ in plants) or from a specific insect tissue.

Tissue culture techniques include use of specific growth media, temperature, and other physical requirements. These techniques enable mass production of near-identical clones, a necessity for biotechnology research and applications.

Having near-identical clones is particularly useful in breeding improved crop and horticultural plants. Current ARS research using tissue culture in improving plant germplasm includes work with alfalfa, sunflowers, oats, forage and turf grasses, cotton, rice, corn, sugar beets, sugarcane, lettuce and other leafy vegetables, sorghum, wheat, and fruit trees such as apples and peaches.

ARS scientists are also using tissue culture to develop reliable laboratory lines of insect cells. The main uses of such cell lines are for study of basic biological factors governing insect growth and metabolism and behavior (so that researchers can manipulate these factors to control pest insects) and for factorylike production of viruses that can be used as biocontrols. Certain constituents of insect viral DNA are being used to produce medical and veterinary vaccines and human hormones in tissue culture.

Increasing profits with tissue-cultured fruit trees

Tissue culture is also a commercially practical means of propagating plants rapidly, uniformly, and free of disease, without climatic and seasonal restrictions. Scientists at the ARS Beltsville Agricultural Research Center in Maryland have solved the major problems of tissue-culturing peach trees. Trees from small shoots raised by tissue culture bore 10 times more peaches in their second year than conventionally grafted trees. Normally, peach trees need 3 years to begin producing a cash crop. But 2-year-old tissue-cultured, own-rooted trees produced 285 peaches per tree, compared with 33 peaches on trees grafted onto seedling root stocks. Eventually, the two types of trees will produce similar yields, but a sizable second-year crop would increase the orchard grower's profits. Beltsville scientists have also overcome the difficulties in getting roots started for tissue culture of apple trees; now focus has shifted to the less difficult but equally important problems of shoot formation.

Gene Transfer

In theory, since a gene is just a piece of DNA—chemical compounds bonded together and arranged in a particular sequence—it can be moved from one organism to another by means of one of many gene-transfer techniques. In fact, ARS scientists have transferred foreign genes into both plants and animals. These genes have produced their particular characteristics (expressed themselves) in the resulting plants and animals and in many of their offspring. The technical difficulties can be enormous, though they are being solved. And in some cases, scientists must use extraordinary precautions to ensure the safety of this research.

The potential payoff is also enormous. One, the years it takes to introduce genetic improvements through traditional breeding could be reduced to a fraction of that time. Two, genes can be transferred not only within species, but among them: An ARS scientist at Beltsville, Maryland, was the first to succeed at microinjecting foreign chromosomes into single plant cells. The result was a petunia with new

drought-tolerance genes that improved the plant's ability to withstand water stress. (Petunias were used for this basic study because they can be grown in the lab from a single cell; see p. 16.)

Researchers are using gene-transfer techniques to enhance effectiveness of biological agents that help control pests of animals, crops, and stored fruits. Other scientists are using gene-transfer techniques to improve the ability of certain food-processing organisms to reduce cholesterol levels in processed foods containing dairy ingredients. And various research projects are using microinjection and other gene-transfer techniques to study and improve wheat, oats, rice, and other cereals. This work is concentrating on resistance to insects such as greenbug, yellow sugarcane aphid, and the Russian wheat aphid; to diseases, especially virus diseases of wheat; and to drought.

Gene transfer can also be used to improve a plant's nutritional quality. For instance, soybeans and other legumes will provide a more complete source of protein when a corn gene enriched in sulfur amino acid content can be engineered into the crops. Legumes are usually very low in sulfur-containing essential amino acids. As a start, ARS scientists at Beltsville, Maryland, in collaboration with scientists from Agrigenetics Basic Science Corporation, found that when one of corn's storage proteins is inserted into tobacco plants, the tobacco seeds synthesize and store the storage protein enriched with sulfur amino acids. Tobacco is being used as a model because scientists haven't yet succeeded in regenerating seed-bearing soybeans from genetically engineered cells (see p. 16).

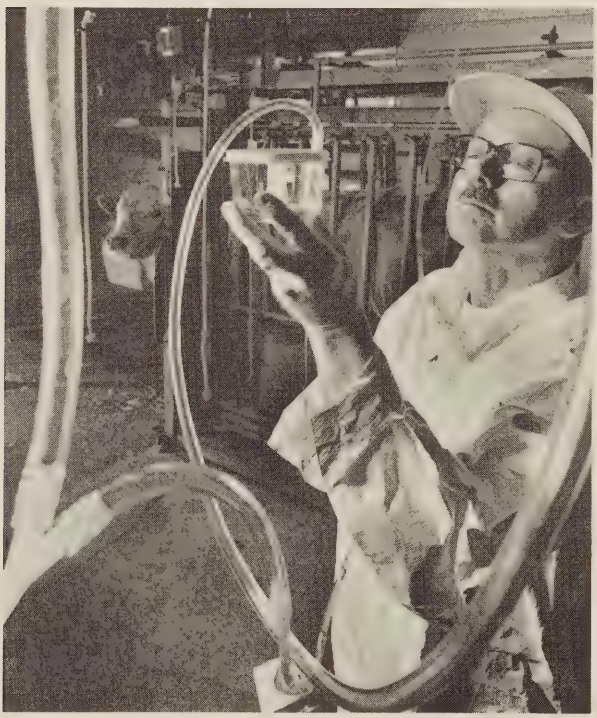
Transgenic plants are more likely than transgenic animals to be commercially available on a large scale in the near future. Gene transfer studies in animals involve ethical considerations not usually applied to plants. But releasing transgenic plants into the environment also raises some concerns that are addressed in careful review case by case.

Shortcuts to breeding better, healthier cows

Finding out, for example, whether a gene inserted into an animal embryo will take hold has to wait on the actual birth. In cattle, the wait is about 280 days. ARS scientists at Beltsville, Maryland, have used a gene-copying system that will cut this waiting period to less than 2 weeks. The new system, successful in mice but designed for future use in cattle, will save genetic engineers a great deal of time. Five to 7 days after scientists have injected a new gene, they will split the embryo into identical twins. One twin embryo will be used for the gene-copying technique, in which as few as 25 of its cells are mixed with the enzyme Taq polymerase. If the injected gene is present, the

Taq polymerase makes a million or more copies of the gene—enough for standard tests to determine its presence. If the result is positive, the scientists will implant the other twin into a surrogate mother cow.

Embryo transplants will also help scientists overcome a big obstacle to breeding disease-resistant cattle—lack of cattle with the same immunity genes for use in research. Today those genes—collectively called the major histocompatibility complex—can be studied in inbred rodents, where rapid reproduction allows relatively quick gene manipulation. Since the same approach in cattle would take 40 to 100 years, Beltsville scientists are using the split embryo technique to decrease the time. Results so far include twin sisters and a bull from the same biological mother with the same genes over most of the histocompatibility complex. When the sisters are bred to the bull, 25 percent of the offspring should have identical genes for immunity. Those offspring will be pioneer sources for cattle with genetic resistance to diseases such as stomach worm, foot-and-mouth disease, and bluetongue.

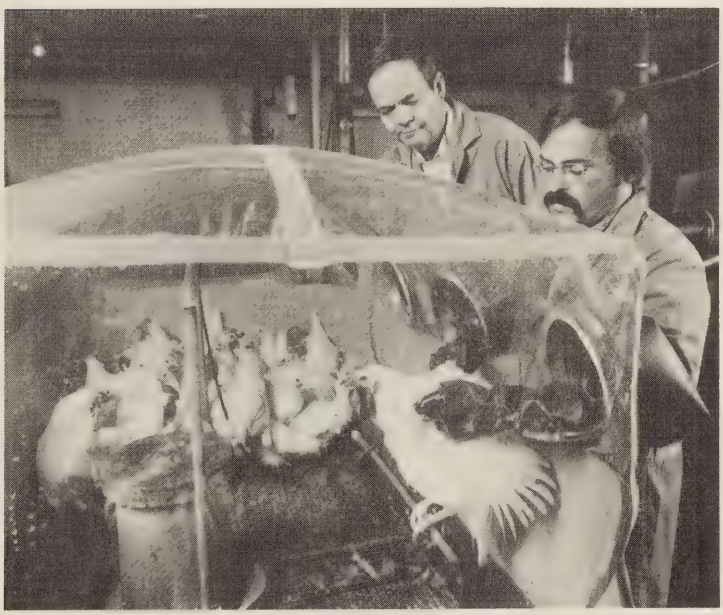


ARS animal physiologist flushes 7-day-old embryos from a cow treated with a hormone that induces multiple ovulation. Recovered embryos are implanted into surrogate mothers or frozen for later use. Ability to store and transplant animal embryos is essential to many gene transfer studies. (88BW0902-4)

A new breed of chickens

Transgenic chickens that can resist avian leukosis virus have been bred by ARS scientists at East Lansing, Michigan. Avian leukosis costs U.S. egg producers $50 to $100 million a year. But it happens that the virus that causes avian leukosis is one of a class of viruses—retroviruses—that can be used to transfer genes. Retroviruses are different because their gene code is contained in their RNA rather than in DNA. Once inside a cell, a retrovirus releases an enzyme that converts its RNA to DNA. The DNA seeks out the cell's genes and merges with them. The cell then produces a new virus particle.

Eventually, retroviruses could be used to transfer genes for various traits: In chickens this could mean less fat, better taste, and ability to grow larger on less feed. But preliminary work has centered on giving chickens an inheritable resistance to the avian leukosis virus itself. Genes were transferred from a strain of the virus that seldom causes avian leukosis. In hundreds of attempts to transfer the virus genes, scientists squirted virus through the eggshell near the day-old embryo and then hatched the egg. Transgenic chickens bred from one of these embryos are defective for virus production and have shown resistance to lab and field strains of the avian leukosis virus through four generations. Ordinarily, the virus commands chicken cells to make more virus particles. But cells in the resistant birds make only the virus envelope, or empty shell surrounding the virus particle.



ARS animal geneticist and assistant check transgenic chickens maintained in isolation under a plastic canopy. (1185X1251—25)

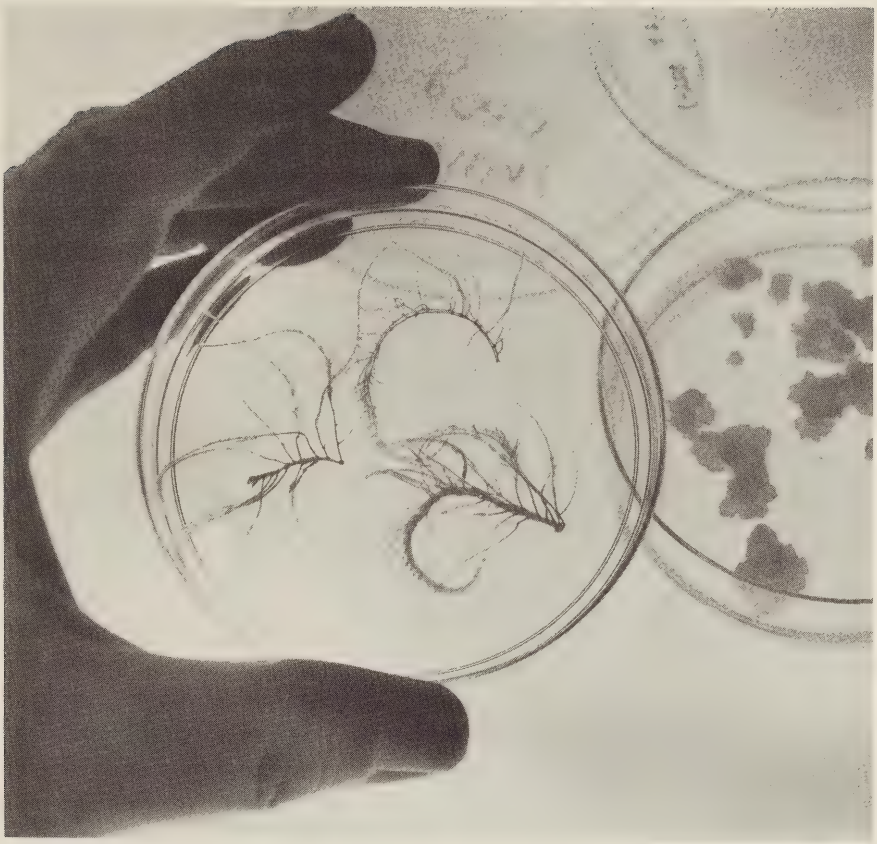
Getting Soybeans To Grow

Geneticists have long since discovered that getting complex plants and animals to grow from single cells in lab dishes is not the simple, unvarying process it was once thought to be. For one thing, many of the genes in any cell of a complex organism are inactive except when they receive a specific stimulus—at certain developmental stages, for example. And strange things happen in the lab dish. Case in point: soybeans.

In the lab dish, a soybean cell's own genes for root and shoot development won't turn on. Without roots and shoots, obviously, there's no soybean plant. And without the ability to grow a whole organism from a genetically engineered cell, there's no point to genetic engineering. This is not an unusual phenomenon; root and shoot genes turn off in other plants too.

But for some plants, like alfalfa, tobacco, and petunias, scientists can just add synthetic hormones, and the roots and shoots develop. (This is one reason these three plants are commonly used in basic experiments with plant genetics.) With soybeans, though, synthetic hormones don't work.

So two ARS scientists at Beltsville, Maryland—a plant physiologist and a geneticist—teamed up to try a different approach to the problem. They knew that a soil organism called *Agrobacterium tumefaciens* causes tumors in plants by inserting genes for both root and shoot formation into the same cell—confusing the plant's growth mechanisms. The researchers inactivated the shoot-forming gene and let the bacterium infect soybean cells in the lab. The resulting root growth proved the technique was workable. Unfortunately, the reverse—inactivating the root-producing gene to get shoots to grow—didn't work in soybean cells. Similar difficulties have been overcome for peach trees (see p. 12), and the scientists are continuing to look for the key in soybeans.



Soybean cells in dish at left have grown roots after a soil organism, *Agrobacterium tumefaciens,* inserted root-producing genes into them. Without added genes, soybean cells grow into unorganized clumps (right). (0687X531-9)

Examining potato plant genetically engineered for insect resistance, ARS plant pathologist (top) finds little damage from Colorado potato beetles (bottom). (0687X534-11/0687X533-22)

Protoplast Fusion

A plant cell wall inhibits exchange of material between cells. Stripping a plant cell of its wall leaves the protoplast, which can then be fused to another protoplast. Scientists use protoplast fusion to produce hybrids between species that cannot be bred conventionally.

Researchers at ARS labs in Beltsville, Maryland, and Philadelphia, Pennsylvania, used protoplast fusion to produce hybrid potato plants with insect resistance built into their leaves. The hybrids contain a rare gene for the insect-repelling chemical leptine, which was transferred into the plants from wild potatoes. Protoplast fusion was used because many wild and cultivated potatoes are sexually incompatible.

Biochemical Engineering

Biochemical engineering is one of the oldest biotechnologies. The biochemist manipulates plant and animal chemistry to make biological factories for everything from cheese and bread and beer and wine to industrial chemicals used in plastics, cosmetics, foods, feeds, detergents, inks, and hundreds of other products. Agrichemicals are important to reducing American dependence on foreign petroleum, and they provide an economically significant nonfood use for crops. In fact, biochemical engineering is a key to enhancing the desirability of American agricultural products both at home and abroad. One ARS project, for example, is to improve whey fermentation—a means to increase demand for dairy products and reduce surpluses. Another project is to improve enzyme production for manufacture of agrichemicals.

Yeast's expanding role as a biotech workhorse

Yeast, a simple one-celled fungus, is a biotechnology tool. It has been used for centuries to convert sugar into carbon dioxide, which makes bread rise, and into the alcohol essential to beer and wine. Yeasts are often used by the food industry in producing soups, soy sauce, and baby food. They are also used to break down lactose and starch, to treat industrial and agricultural waste, and to produce vitamin supplements and ethanol fuel. The ARS Northern Regional Research Center in Peoria, Illinois, maintains a research collection of 14,200 yeast strains. So far, not many of these strains are used commercially.

In the past, commercial interest in expanding the use of yeasts may have been held back by limited knowledge of their biochemistry. But research in the molecular biochemistry of yeasts has increased basic understanding of their metabolic processes to the point that scientists

are beginning to envision ways of creating marketable products inexpensively through fermentation, using genetically engineered yeasts.

From hundreds of yeast strains in the Peoria collection, researchers turned up four that could become modern biotechnology tools because they contain strings of DNA called linear plasmids. Currently, genetic engineers use circular plasmids as vehicles for transferring genes from one microorganism to another. In filamentous fungi, corn, and other higher plants, linear plasmids are surrounded by membranes of cell structures such as mitochondria and nuclei; these membranes are obstacles to successful gene transfer. Linear plasmids found in the four strains of yeast, on the other hand, replicate and float freely within the cell's inner space, or cytoplasm.

Linear plasmids have been found in one other yeast, *Kluyveromyces lactis,* by Japanese scientists. In 1986, British scientists used *K. lactis* linear plasmids to shuttle genes to another yeast in a successful gene transfer experiment. ARS geneticists and colleagues with the Biotechnology Research and Development Corporation of Peoria are carrying out similar experiments with the four Peoria yeast strains and are screening other yeasts in the collection for other plasmids to serve as possible biotech tools. Unlike *K. lactis,* which was already in commercial use before discovery of the linear plasmids, the four Peoria strains had no previously known economic potential.

Improving efficiency of commercial cucumber fermentations

During cucumber fermentation, bacteria used to convert sugars to lactic acid also produce carbon dioxide from malic acid of the cucumbers. Unless the carbon dioxide is purged from the fermenting brine, bloater damage (hollow cucumbers) can result. ARS researchers in Raleigh, North Carolina, have developed cultures of lactic acid bacteria that can't produce carbon dioxide from the malic acid. The scientists chemically mutated, selected, and improved strains of the bacterium, *Lactobacillus plantarum,* that had been isolated from commercial fermentations. ARS is cooperating with Chr. Hansen's Laboratory, of Milwaukee, which has been licensed to grow the new cultures to test feasibility of using them commercially. Pickle Packers International and its member companies have supported this research and are eager to use the new cultures.

The Plant Gene Expression Center

The Plant Gene Expression Center in Albany, California, was established in 1984 as a joint venture of the Agricultural Research Service, the University of California at Berkeley, and the California Agricultural Experiment Station. The center is in the San Francisco Bay area, a region that has one of the highest concentrations of plant genetic engineering research in the United States.

Research at the center is designed to provide new, precise, and much-needed information on exactly how plant genes are regulated, or expressed, in crop plants, on how instructions contained in a gene are executed by the plant. With this information and the tools of modern biotechnology, plant geneticists will be better able to isolate and transfer the genes that control agriculturally important traits. This research may also provide information critical to enhancing nonfood uses of the plant, such as improving the quality of the fiber or oils the plant provides.

Scientists at the center are engaged in several research projects that delve into the basic genetic mechanisms in plants. Two typical projects are—

- Learning how phytochrome controls genes. Phytochrome is a light-detecting molecule that was discovered in plants by ARS scientists some 35 years ago. It can be thought of as a powerful central switch that turns on genes that regulate some of the processes most vital to agriculture, including seed germination, flowering, and formation of chloroplasts (the structures in which photosynthesis takes place). Ability to regulate the activity of phytochrome could lead to increased energy production in the plant, which would increase its productivity.
- Studying the hormones and genes that regulate growth and aging in plants. Current research is concentrating on the growth-regulating hormone auxin, which in turn controls production of another hormone, ethylene. Ethylene causes fruit to ripen and eventually spoil. Almost 50 percent of the fresh fruits and vegetables harvested in the United States are eventually lost because of spoilage caused by ethylene. The long-term objective of this research is to be able to regulate the genes responsible for natural production of ethylene and therefore to control the ripening process.

Bioregulation

Bioregulators are chemicals, mainly hormones and enzymes, that control reproduction in insects and growth and development, behavior, reproduction, and metabolic functions such as photosynthesis in plants. ARS researchers are using several biotechnology tools in developing means to control and adjust some of these activities to improve plant and animal reproductive efficiency, tolerance to stress, growth rate, resistance to pests and diseases, control of pest insects, and timing of biological events such as fruit ripening and budbreak in fruit trees.

Typical of ARS bioregulation research are work on—

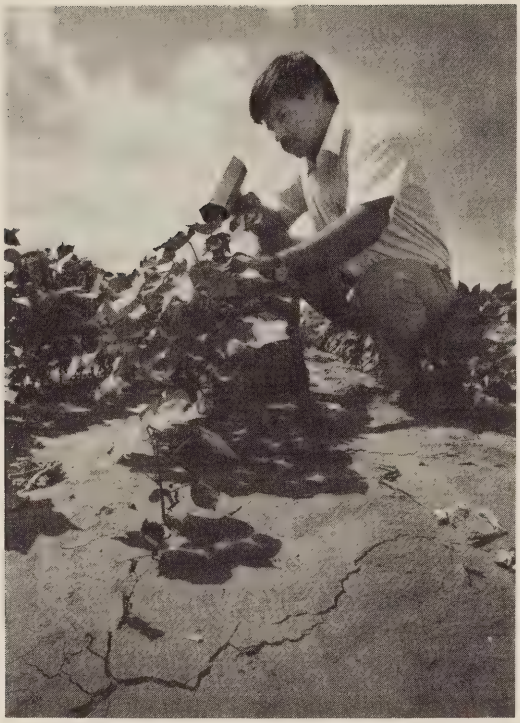
- Hormones governing brain/pituitary/gonad interrelationships in pigs; the objective is to adjust irregularities in swine reproductive cycles that are costly to farmers.
- Hormonal regulation of growth and development in plants. One such study is focusing on auxin, a growth-enhancing hormone. Another study is concentrating on the hormones that regulate development of wheat spikes and seed. Greater understanding of the genetic and biochemical factors involved in growth and development will lead to improved varieties.
- Hormonal regulation of insect reproduction and biochemistry of insect communication, chiefly the production of mating attractants. Disrupting their reproduction is a primary means of controlling insect pests. Other research focuses on the role of peptides as bioregulatory chemicals in livestock insects.
- The biochemical mechanisms of molecular transport across membranes—leading to an understanding of how sugars are moved from one part of a plant to another; ability to control these mechanisms will help improve quality and value of crop plants.
- Role of phytoalexins and other substances in resistance of citrus plants to nematodes. Phytoalexins are among the biologically active natural products that scientists are investigating as potential natural pesticides. Transferring the genes that govern manufacture of these products is a possible means of imparting this ability to other plants.
- Mechanisms that produce sterility in the progeny resulting from cross-breeding the tobacco budworm with a related species. Advances in this area of research may lead to the ability to induce male sterility in pest insect species (for which simple cross-breeding is impractical) via direct genetic intervention.

- Hormonal regulation of growth and development in pest insects (for example, the face fly and the corn earworm and tobacco budworm). One research project is focusing on synthesis of chitin, which forms the insect cuticle, or skeleton; disrupting this process would be an effective control measure.
- Microbial production of toxins (such as aflatoxins); aim is to find ways to mitigate this food-safety problem.
- Enzymes that govern photosynthetic carbon dioxide fixation so that the appropriate genetic factors can be included in breeding programs for increased crop productivity. Researchers also expect that the information gained in these studies will enable them to use genetic or biochemical engineering to modify the processes that limit photosynthetic rates.
- Regulation of photosynthetic carbohydrate metabolism in plants. The overall objective of this research is to increase the photosynthetic efficiency of economically valuable plants by manipulating the biochemical mechanisms that regulate starch/sucrose formation in leaves.
- The plant growth regulators abscisic acid, gibberellic acid, and jasmonic acid and enzymes that in turn regulate the regulators. Related research focuses on how light (especially photoperiod—the ratio of light to darkness in a given day) influences the development of flowering in plants. Understanding these regulatory mechanisms should lead to improved varieties.
- Biologically active natural products from microorganisms. These compounds, which are naturally occurring chemicals and are readily biodegradable, have a range of uses. Some function as herbicides, some as plant growth regulators. Others control organisms that cause plant diseases. Still others have potential as pharmaceuticals and insect-control agents.

Understanding bioregulation in plants

Several noteworthy recent advances in research on plant bioregulation have come from ARS labs.

- At Lubbock, Texas, scientists have made a key discovery about how plant enzymes respond to various temperatures. They found that the enzymes operate most efficiently within narrow temperature ranges called thermal kinetic windows. The temperature window varied among the five species studied—wheat, corn, cotton, spinach, and cucumbers. Field tests are continuing, and other enzymes will be studied. Transferring one plant's genes for controlling an enzyme's temperature response



In research on thermal kinetic windows, a factor in a plant's ability to tolerate heat and drought, an ARS plant physiologist uses an infrared thermometer to determine foliage temperatures of heat- and drought-stressed cotton plants. (88BW1520-10)

into other plants may change those plants' thermal kinetic windows. That could lead to new crop varieties that grow better at higher—or lower—temperatures than they do now.

- Studies in the lab at Aiea, Hawaii, have shown that when clusters of corn cells are subjected to too much salt or too little water, the cells will manufacture three proteins not found in other cells free of such stresses. Likewise, when corn seedlings were deprived of water, they reacted by synthesizing two other proteins that were not produced by their well-watered counterparts. Those two proteins rapidly disappeared when the plants were watered. If the genes that code for these proteins can be isolated, scientists hope to genetically engineer more tolerant varieties of corn as well as transfer the tolerance genes into other plants.
- Researchers at Urbana, Illinois, have isolated and cloned a gene responsible for the enzyme rubisco activase. This enzyme helps plants adjust to changes in light intensity during the first step in photosynthesis—converting atmospheric carbon dioxide to sugar. Now scientists can transfer modified versions of the cloned gene into chloroplasts (part of the plant cell that contains chlorophyll) to see which versions work best in different environments.

- Scientists at Raleigh, North Carolina, have purified and characterized the enzyme that determines oil content in soybeans and have produced the first soybean containing half the normal level of saturated fat. Research has also led to development of high-yielding high-protein soybeans. When these soybeans become commercially available, farmers will have a much-needed choice of which to grow—high-oil or high-protein varieties—depending on market conditions.
- Bringing genetic engineering of oilseed crops a step closer to reality is the synthesis by ARS scientists at Peoria, Illinois, of a gene that carries the blueprint for a key protein required for producing plant oil. The scientists have successfully inserted the new gene into a common bacterium, *Escherichia coli*. Once in the bacterium's genetic makeup, the gene directs production of acyl carrier protein. In plants, this protein is needed for several steps in the synthesis of fatty acids that are components of cell membranes and of oils stored in plant seed. Now enough of the protein can be made and purified to thoroughly study plant fatty acid synthesis. Forming the synthetic gene for acyl carrier protein was made easier by a new laboratory instrument called a DNA synthesizer, which links nucleotides into the proper sequence for construction of a particular gene. In followup work, the Peoria scientists have joined the gene for acyl carrier protein to a bacterial gene. This new gene has produced a fusion protein with properties of both the acyl carrier and bacterial proteins. Potential applications include improved methods for purifying other proteins used in oil production. Another possibility: If the engineered gene can be put into plants, the unique properties of the fusion protein could enable changes in plant oil production.
- At Beltsville, Maryland, two ARS plant pathologists have discovered that induced resistance to alfalfa anthracnose disease is controlled by specific genes. Scientists first found induced resistance in plants over 40 years ago and in alfalfa in 1984, but only lately have they been able to explain some of its mechanics. Molecules on a fungus that causes alfalfa anthracnose disease act like an antigen on an animal pathogen. These molecules—elicitors—trigger an antibodylike response in the plants. The plant pathologists have isolated the antifungus response compounds, which are called phytoalexins. Like antibodies, phytoalexins are not produced until infection occurs, and their production is ordered by certain genes. The alfalfa discovery has led the two scientists to develop the first rapid screening test to find and measure the effects of phytoalexins. They use pieces of

alfalfa tissue growing in laboratory dishes to trace the events of induced resistance. They want to know just how a plant recognizes that a disease organism is penetrating its surface. To find out, they are isolating the elicitors from the fungus and then using DNA probes to find the proper plant genes that respond to them. What studies of induced resistance in alfalfa reveal could lead to genetic engineering of many crops for strong, built-in defenses.

Bioregulation of human response to nutrients

Most ARS research on human nutrition is aimed at finding out how the body responds to and uses vitamins, minerals, and other nutrients. Working with rats, for example, scientists at the ARS Human Nutrition Research Center on Aging at Tufts in Boston have isolated the two genes that produce messenger RNA's that contain the information on how to make each of the subunit proteins of ferritin, the iron-storage protein. These two messenger RNA's also bear a sequence that repressor proteins bind to; the repressor proteins restrict the amount of ferritin being made when the cell is not receiving iron. When iron enters the cell, the repressor protein dissociates from the messenger RNA's, allowing them to make more ferritin to regulate the accumulation of iron. Further research is underway with the aim of eventually determining whether the accumulation of iron in the body contributes to the aging process in humans.

As part of studies on induced resistance in plants, ARS plant pathologist inoculates young alfalfa sprouts with a solution of harmful fungus spores. (0687X526-23)